

Amdt. dated February 8, 2010

Reply to Office Action of October 6, 2009, the response to
which is hereby extended by one month to February 6, 2010
by the attached Petition to Extend Time and requisite fee

Amendments to the Specification:

Please replace the first paragraph of page 1 with the following amended paragraph:

The present invention relates to methods of screening for antifungal agents having the activity of inhibiting **glycosylphosphatidylinositol synthase** (GPI synthase), which is involved in the synthesis of fungal cell walls.

Please replace the second paragraph of page 1 with the following amended paragraph:

The present inventors noticed that adhesion to host cells is important for fungi to exert their pathogenicity, and that adhesion factors involved in fungal cell adhesion are transported to the surface layers of cell walls after glycosylphosphatidylinositol (GPI) anchors on the cell membrane (Non-Patent Document 1: Hamada K *et al.*, Mol. Gen. Genet., 258: 53-59, 1998). Accordingly, the present inventors considered that novel antifungal agents that inhibit the synthesis of fungal cell walls and also inhibit the adhesion of fungal cells to host cells could be generated by inhibiting the process of transporting proteins anchored with **glycosylphosphatidylinositol GPI** (GPI-anchored proteins) to cell walls. Thus, the present inventors started study.

Please replace the first full paragraph of page 2 with the following amended paragraph:

The inventors then found that the GWT1 gene product (hereinafter referred to as "GWT1 protein") has the activity of synthesizing **glucosaminyl-acylphosphatidylinositol** (GlcN-(acyl)PI) by transferring an acyl group to GlcN-PI in the GPI biosynthesis pathway (Fig. 1; Kinoshita and Inoue, Curr Opin Chem Biol 2000 Dec;4(6): 632-8; Ferguson *et al.*, Biochim Biophys Acta 1999 Oct 8; 1455 (2-3): 327-40). The inventors conceived that compounds inhibiting the synthesis of fungal cell walls could be found by screening for compounds that inhibit this activity, and thus completed the present invention.

Please replace the second full paragraph of page 3 with the following amended paragraph:

The proteins and protein mutants prepared using the above-described hybridization techniques normally have high homology (for example, 60% or higher, 70% or higher, 80% or higher, 90% or higher, or 95% or higher homology) to proteins consisting of the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, or 14 at the amino acid level. The amino acid sequence homology can be determined using a BLASTx program (at the amino acid level; Altschul *et al.*, J. Mol. Biol. 215:403-410, 1990). This program is based on the BLAST algorithm of Karlin and Altschul (Proc. Natl. Acad. Sci. USA 87:2264-2268, 1990; Proc. Natl. Acad. Sci. USA 90:5873-5877, 1993). When the amino acid sequences are analyzed using **BLASTX**, parameters of, for example, score= 50 and wordlength= 3 are used. Alternatively, when using the Gapped BLAST program, the amino acid sequences can be analyzed by the method described by Altschul *et al.*

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(Nucleic. Acids. Res. 25:3389-3402, 1997). When the BLAST and Gapped BLAST programs are used, the default parameter values for each program are used. Specific procedures for these analyses are known in the art (<http://www.ncbi.nlm.nih.gov>).

Please replace the third full paragraph of page 5 with the following amended paragraph:

S. cerevisiae cells to which the GWT1 gene are introduced are cultured while shaking in an appropriate medium, such as SD(ura-) liquid medium, at an appropriate temperature, for example 24°C. The fungal cells are harvested in the middle logarithmic growth phase. After being washed with TM buffer (50 mM Tris-HCl (pH 7.5) and 2 mM MgCl₂), the fungal cells are suspended in an adequate amount (for example, 2 ml) of TM buffer + protease inhibitor (Complete™, protease inhibitor, Roche). An adequate amount (for example, 1.5 ml) of glass beads is added to the suspension. The samples are vortexed and placed on ice, and these procedures are repeated (for example, ten cycles of vortexing for 30 seconds and placing on ice for 30 seconds) to disrupt fungal cells.

Please replace the second full paragraph of page 9 with the following amended paragraph:

Wild-type *S. cerevisiae* strain, the GWT1 gene-deficient strain Δgwt1, and the strain Δgwt1 into which the GWT1 overexpression plasmid was introduced were each cultured in 100 ml of YPD medium shaken at 24°C, and then harvested in the middle logarithmic growth phase (OD₆₀₀= 1 ~ 3). The fungal cells were washed with TM buffer (50 mM Tris-HCl (pH 7.5) and 2 mM MgCl₂), and then suspended in 2 ml of TM buffer + protease inhibitor (1 tablet of Complete™, protease inhibitor, (Roche) / 25 ml). 1.5 ml of glass beads was added to the suspension. The mixture was vortexed for 30 seconds, and then placed on ice for 30 seconds. These procedures were repeated ten times to disrupt the fungal cells. The cell homogenate was transferred into a new tube, and centrifuged at 1000 g at 4°C for five minutes to precipitate the glass beads and undisrupted fungal cells. The supernatant was transferred to another tube, and centrifuged at 13 000g at 4°C for 20 minutes to precipitate the membrane fraction comprising organelles (total membrane fraction). The resulting precipitate was used as the membrane fraction.

Please replace pages 1 - 31 of the Sequence Listing with pages 1 - 31 of the Substitute Sequence Listing submitted herewith.